

ORIGINAL RESEARCH ARTICLE

A trabeculae-like biomimetic bone-filling material as a potential organoid for bone defect treatment

Supplementary File

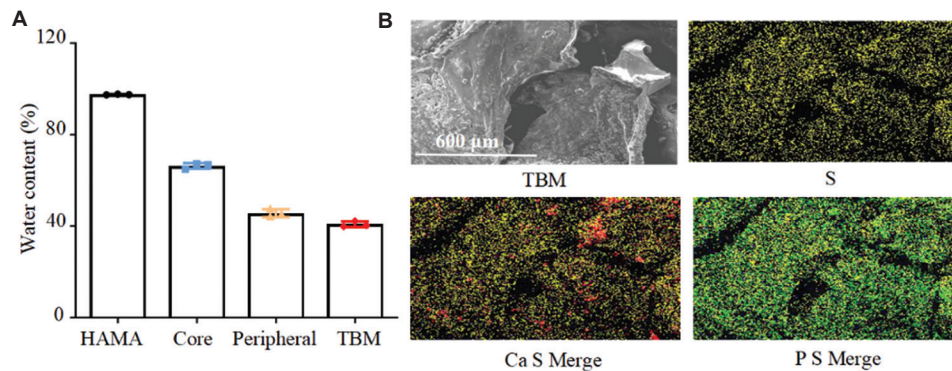


Figure S1. The characterization of the trabeculae-like biomimetic bone filling material (TBM). (A) The water content in the hyaluronic acid methacryloyl (HAMA), the HAMA-embedded core pillar (Core), the HAMA-embedded peripheral porous framework (Peripheral), and the TBM. (B) Energy-dispersive spectroscopy mapping of the TBM (scale bar: 600 μm ; magnification: $\times 35$).

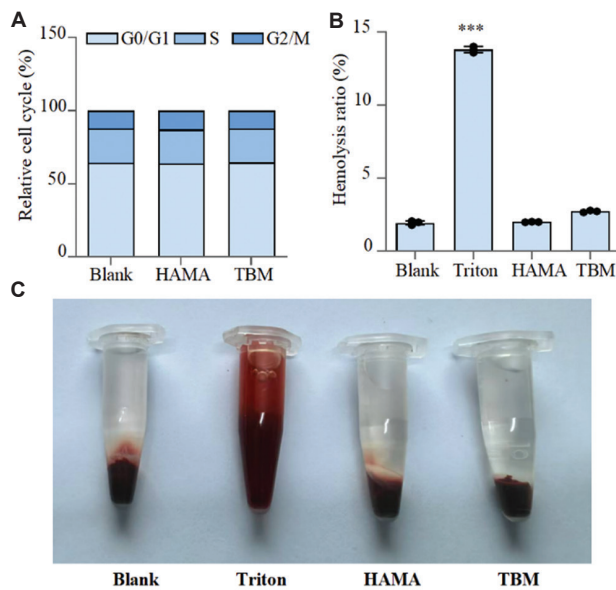


Figure S2. The cytocompatibility of the trabeculae-like biomimetic bone filling material (TBM). (A) The cell cycle of hyaluronic acid methacryloyl (HAMA) and TBM. (B and C) The hemolysis rate of HAMA and TBM. Note: *** $p < 0.001$.

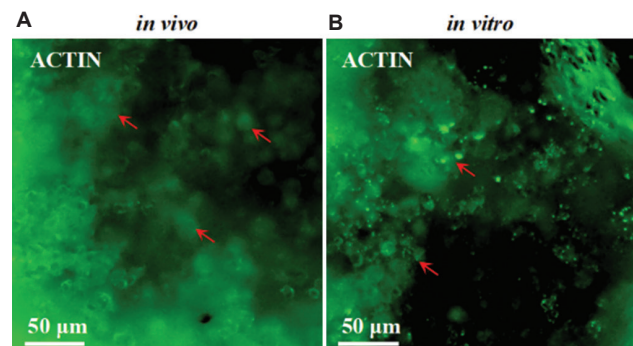


Figure S3. The expression of ACTIN in the trabeculae-like biomimetic bone filling material (TBM) embedded with mouse adipose-derived mesenchymal stem cells (ADSCs). (A) The expression of ACTIN in the TBM embedded with ADSCs as detected by immunofluorescence staining (scale bar: 50 μm ; magnification: $\times 190$). Red arrows are pointing the ADSCs upon the porous skeletal structure. (B) The expression of ACTIN in the organoid implanted *in vivo* for 10 days as detected by immunofluorescence staining (scale bar: 50 μm ; magnification: $\times 190$). Red arrows indicate ACTIN expression in cells.

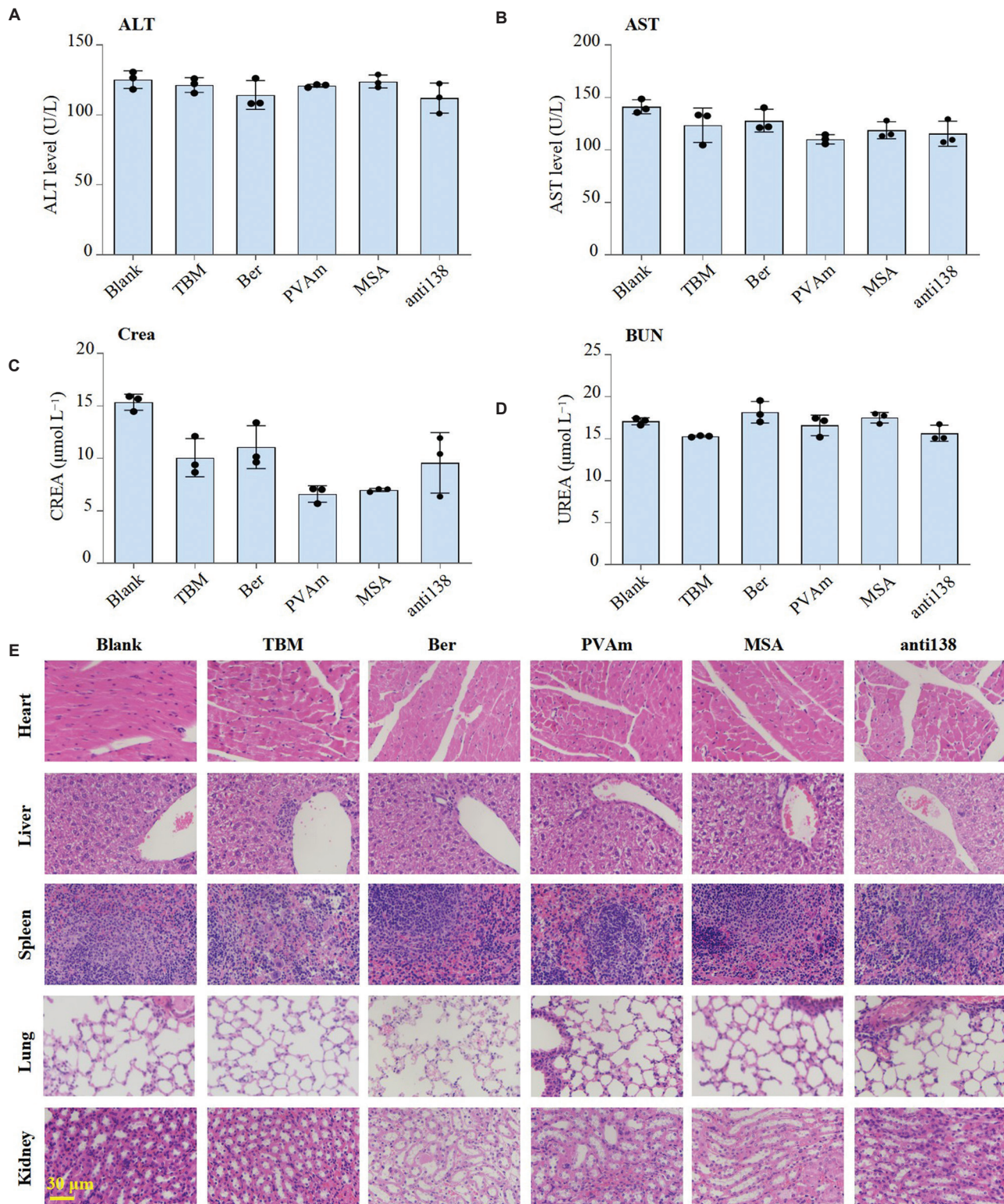


Figure S4. The biocompatibility of the trabeculae-like biomimetic bone filling material (TBM) in tibial defect mice. (A-D) The levels of alanine transaminase (ALT; A), aspartate aminotransferase (AST; B), creatinine (Crea; C), and blood urea nitrogen (BUN; D) in blood serum of mice (data represented as mean \pm SD, $n = 3$). (E) The hematoxylin-eosin staining of the heart, liver, spleen, lung, and kidney (scale bar: 30 μm ; magnification: $\times 160$). Notes: Blank: No treatment for the fracture region; TBM: TBM filling the fracture region; Ber: TBM loaded with bergamottin filling the fracture region; Polyvinylamine (PVAm): TBM loaded with PVAm filling the fracture region; MSA: TBM loaded with empty recombinant tRNA (loaded by PVAm) filling the fracture region; anti138: TBM loaded with recombinant miR-138-5p antagonist (loaded by PVAm) filling the fracture region.

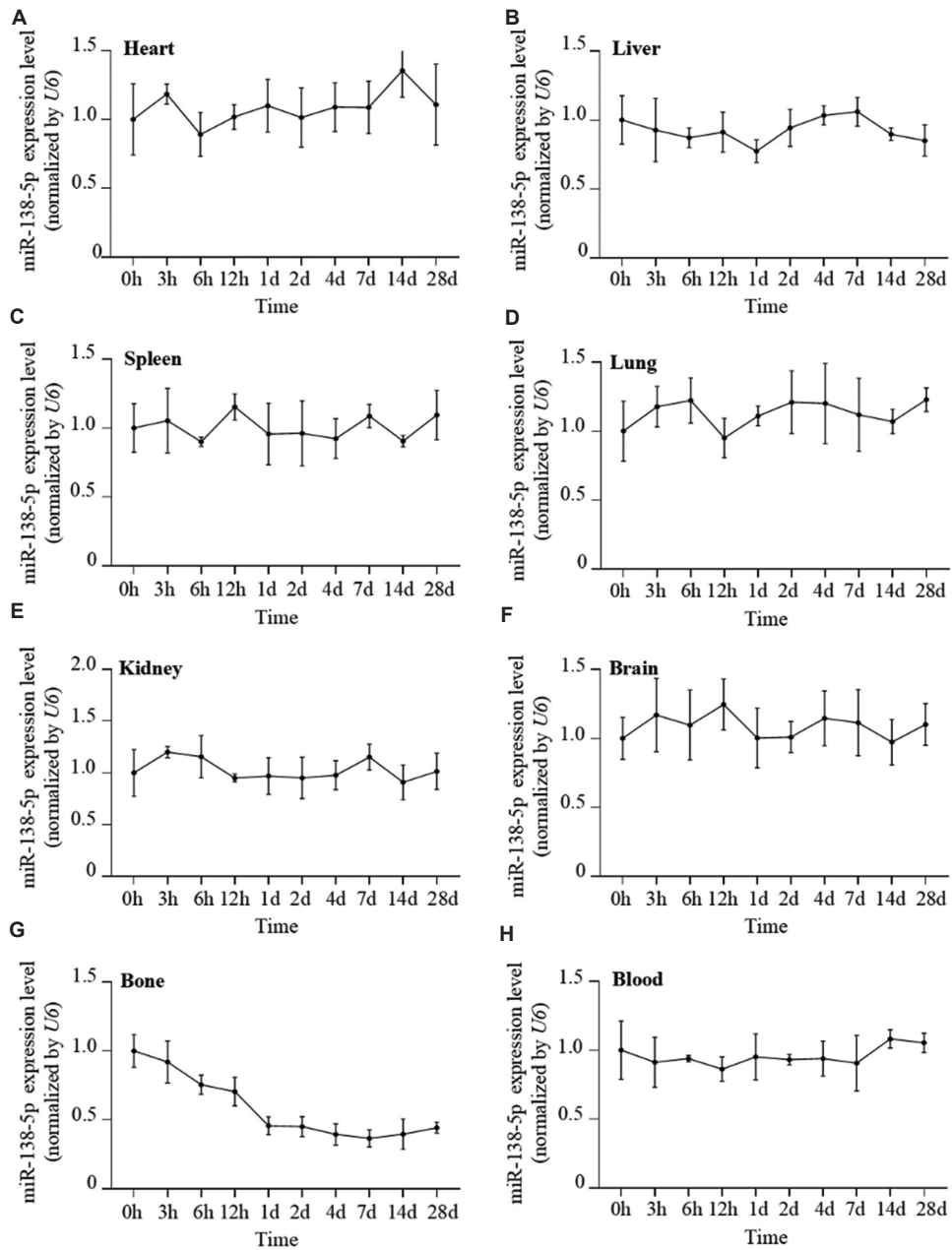


Figure S5. Pharmacokinetics of the trabeculae-like biomimetic bone filling material (TBM) in tibial defect mice. (A-H) The expression levels of miR-138-5p in different organs of tibial defect mice at the specified intervals after TBM treatment, as detected by reverse transcription-polymerase chain reaction (data represented as mean \pm SD, $n = 3$).

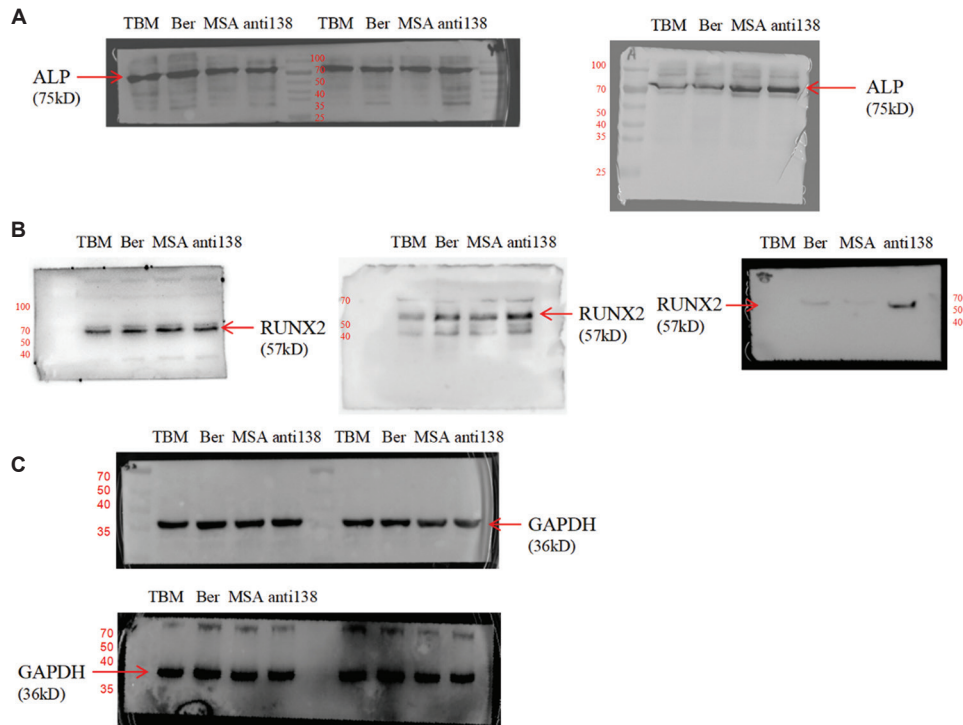


Figure S6. The original images for western blotting (as in Figure 4I) for alkaline phosphatase (ALP), runt-related transcription factor 2 (RUNX2), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in mouse adipose-derived mesenchymal stem cells (ADSCs) cultured in TBM. (A) Western blotting for ALP (75 kDa). (B) Western blotting for RUNX2 (57 kDa). (C) Western blotting for GAPDH (36 kDa).

Notes: TBM: Blank TBM; Ber: TBM loaded with bergamottin; MSA: TBM loaded with empty recombinant tRNA; anti138: TBM loaded with the recombinant miR-138-5p antagonist.